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Fungicidal effect of plant extracts on the growth of *Sclerotium rolfsii,* the incitant of collar rot of chickpea

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Abstract
Sclerotium rolfsii is an important ubiquitous and polyphagous soil borne pathogen, known to cause collar rot of chickpea. This is a serious and well spread disease in Uttar Pradesh. An *in vitro* experiment was conducted to evaluate the best botanical effective against *Sclerotium rolfsii* by using food poison technique. Two concentrations of each botanical were maintained. Out of six different medicinal phytoextracts viz. Onion bulb (*Allium cepa*), Zinger rhizome (*Zingiber officinale*), Bael leaf (*Aegle marmelos*), Lemon grass leaf (*Cymbopogon citrates*), Datura leaf (*Datura stramonium*) and Calotrophis leaf (*Calotropis procera*) screened, Lemon grass leaf (*Cymbopogon citrates*) showed greater effect in reducing the pathogen growth by 59.62 and 71.66% at 10% and 15% concentrations respectively followed by Datura leaf (*Datura stramonium*) and Calotrophis leaf (*Calotropis procera*). Least inhibition percent was noticed with Onion bulb (*Allium cepa*) with only 28.77% and 39.77% respectively. Thus, of all the tested botanicals, Lemon grass at 15% concentration was found to be highly effective in suppressing collar rot disease caused by *Sclerotium rolfsii.*

Keywords: *Sclerotium rolfsii*, collar rot, botanicals, phyto extracts, chickpea

Introduction
Pulses (grain legumes) are the second most important group of crops worldwide. Globally, 840 million people are under nourished mainly on account of inadequate intake of proteins, vitamins and minerals in their diets (Chakraborty and Mondal, 2015) [3]. The major pulse crops those have been domesticated and are under cultivation are black gram, chickpea, cowpea, mung bean, lentil, moth bean, pea, pigeon pea etc. Among them, Chickpea ranks first in overall pulse production of India. . Hence, India is the leading producer in the world. About 80% of world chickpea is produced from southern Asia and south west Asian regions. Chickpea accounts to almost 45% of total pulses produced in India and also 75% of the world's chickpea is produced from India (Maurya and Kumar, 2018) [6]. Chickpea is frequently subjected to various crop losses because of diseases and pests varying from 5-10% and 50-100% in temperate and sub-tropical regions (Van Emden *et al*. 1988) [12]. At present chickpea is infected with 172 pathogens, among them 67 are fungi, 3 are bacteria, 22 mycoplasmas and viruses along with 80 nematodes. India had reported maximum number of diseases (almost 40) compared to other countries. Widely distributed pathogens are *Ascochyta rabiei*, *Fusarium oxysporum* f.sp. *ciceri*, *Uromyces ciceris arietini*, *Razactonia bataticola*, *Sclerotium rolfsii*, Cucumber mosaic virus (Nene *et al.* 1996) [8]. Among them, *Sclerotium rolfsii* is a serious pathogen causing collar rot of chickpea noticed to be infecting major parts of Uttar Pradesh. Collar rot of chickpea is well known and wide spread disease in India. About 2-5% of losses are caused every year which may even reach up to 60% under severe conditions. It was reported that 54.7 – 95% of mortality occurred in chickpea seedlings because of collar rot disease (Mathur and Sinha, 1968) [5]. It is predominant in tropical and sub-tropical regions where high temperatures prevail during monsoons. Presently, 500 plant species belonging to 100 families were reported to be infected with this pathogen (Aycock, 1966) [1]. Almost 10% of grain loss is reported due to this disease (Nene and Reddy, 1987) [7].
Chemical fungicides are not so effective against *Sclerotium rolfsii*, as it is soil borne and systemic in nature. Most of them leave toxic effect to plants and soil in the form of residues.

Botanicals are of natural origin, biodegradable and non-toxic to environment. Considering the nature safety, cost effective and host target specificity, present investigation was carried out to study the *in-vitro* effect of various phyto extracts viz. Onion bulb (*Allium cepa*), Zinger rhizome (*Zingiber officinale*), Bael leaf (*Aegle marmelos*), Datura leaf (*Datura stramonium*), Calotrophis leaf (*Calotropis procera*) and Lemon grass leaf (*Cymbopogon citrates*) at two different concentrations (10% and 15%) against *Sclerotium rolfsii* infecting collar rot of chickpea and thus improving the yield of chickpea in Uttar Pradesh.

Materials and Methods

Isolation of pathogen

The root samples showing typical symptoms were collected from Agriculture Farm, BHU and packed in polythene bags and sealed. They were bought to laboratory for isolation of pathogen. Collected disease roots were first sterilized with ethyl alcohol using cotton swab. Later they were cut into small pieces of 3 mm^2 size by using sterile scalpel. They are surface sterilized by dipping in 0.1 per cent mercuric chloride solution for 30 sec. Then immediately rinse them in three changes of sterilized distilled water to remove the traces of mercuric chloride. Allow to air dry it by placing on sterilized filter paper and then transfer them on to PDA plated petri dishes using forceps. Inoculated plates were incubated in B.O.D incubator at 28 ± 2 °C by providing favorable conditions for growth of pathogen. Cultures were purified by using hyphal tip method. It was done by picking up pure hyphal structure by using low power of the microscope and carefully transferring to fresh PDA petri dish and maintained at 25 ± 2 ℃ for 10 days. After purifying the infected fungus, their morphological and cultural characters such as color, size, growth rate, type of mycelium were recorded under microscope for their identity. By comparing with available standard literature, pathogen was identified as *Sclerotium rolfsii* (Barnett and Hunter, 1972) [2].

Evaluation of phyto extracts on mycelial growth of *Sclerotium rolfsii*

Plant materials

Antifungal activity of various medicinal phyto extracts were experimented under laboratory conditions against *Sclerotium rolfsii.* Extracts from locally available six different plants were selected *viz.* Onion bulb (*Allium cepa*), Zinger rhizome (*Zingiber officinale*), Bael leaf (*Aegle marmelos*), Datura leaf (*Datura stramonium*), Calotrophis leaf (*Calotropis procera*) and Lemon grass leaf (*Cymbopogon citrates*) and tested against the pathogen. PDA was used as control. Two concentrations *viz*. 10% and 15% were prepared for each treatment.

Extraction

Preparation of plant extracts was simple and easy. To prepare 10% of phyto extract, 10 gms leaves (bulb in case of onion and rhizome in case of zinger) of the medicinal plant were taken in 100 ml of distilled water and allowed to boil them until it becomes soft and for 15% take 15 gms of leaves in 100 ml of water. Later this softened material was crushed by using mortar and pestle. The obtained extracts were allowed to filter through whatman No.1 filter paper. Clear plant extract which was filtered is boiled by adding two grams of dextrose and agar. Finalize the volume to 100 ml by adding distilled water. Autoclave it at 15 lbs pressure for 20 minutes. Just before pouring add a pinch of streptomycin sulphate in each flask to avoid bacterial contamination. Pour 20 ml of PDA in each petri plate and allow it to solidify. Cut a disc of 5 mm *S. rolfsii* culture from 4 days old plate and place them on to fresh PDA place. Three replications were maintained for each treatment along with a control. Incubate them at 27 ± 2 °C in the BOD incubator. Note down the readings at regular intervals by comparing with control. Calculate the inhibition percentage of different botanicals over control with given this formula:

$$I = \frac{C - T}{C} \times 100$$

Where,
I = Per cent reduction in growth of *S. rolfsii*
C = Radial growth (mm) in control
T = Radial growth (mm) in treatment

Results and Discussion

Inhibition rate of pathogen was tested using various phyto extracts extracted from various parts of the plant. Selected phyto extracts were Onion bulb (*Allium cepa*), Zinger rhizome (*Zingiber officinale*), Bael leaf (*Aegle marmelos*), Datura leaf (*Datura stramonium*), Calotrophis leaf (*Calotropis procera*) and Lemon grass leaf (*Cymbopogon citrates*) which were locally available in university campus. Calculations were noted by maintaining PDA as control. Effects of botanicals were experimented with two different concentrations *viz*. 10% and 15%. The recorded inhibition rate is tabulated in Table 1.

The data undertaken clearly depicts that *Lemon grass leaf* (*Cymbopogon citrates)* was found best botanical in suppressing the pathogen at 10% and 15% concentrations (59.62 and 71.66% respectively). Datura leaf (*Datura stramonium*) also showed greater effect following Lemon grass with 58.88 and 63.33% at 10% and 15% respectively. Least percent of inhibition was recorded with Onion bulb (*Allium cepa*) and Zinger rhizome (*Zingiber officinale*) resulting only below 40% and approximately 40% of inhibition respectively. Moderate rate of pathogen suppression was observed in case of leaf (*Calotropis procera*) and Bael leaf (*Aegle marmelos*). Inhibition of pathogen growth might be due to the action of antifungal activity of extracted plant parts that helped in reducing the growth of fungus.

The obtained inhibition rates of botanicals also found confirmative with the findings of Handique and Singh (1990) [4] where they concluded that Lemon grass leaf (*Cymbopogon citrates)* obtained 80% inhibition rate when used at 1000 ppm. Also the findings of Shivapuri *et al.* (1997) [11] visualized that Onion bulb (*Allium cepa*) is least in inhibiting *S. rolfsii*. He found only 10.44% of pathogen suppressed when compared to control. Several other scientists declared that plant extracts significantly inhibit the fungus growth because of their antifungal activity (Sab *et al.* 2014 [9] and Sana *et al*. 2016) [10]

Table 1: *In vitro* evaluation of different phyto extracts on mycelial growth of *Sclerotium rolfsii*

S. No.	Phyto extracts	Radial growth (cm)		Percent Inhibition (%)	
		10%	15%	10%	15%
1	Onion (*Allium cepa*)	6.41	5.42	28.77	39.77
2	Zinger (*Zingiber officinale*)	5.30	4.86	41.11	45.9
3	Lemon grass (*Cymbopogon citrates*)	3.63	2.55	59.62	71.66
4	Calotrophis (*Calotropis procera*)	4.40	3.60	51.11	60.00
5	Datura (*Datura stramonium*)	3.70	3.30	58.88	63.33
6	Bael (*Aegle marmelos*)	4.83	3.95	46.29	56.07
7	Control	9.00	9.00	0.00	0.00
		Phyto extracts	Concentration	Phyto extracts × Concentration	
	SEm ±	0.394	0.131	0.788	
	CD at 5%	1.151	0.384	2.302	

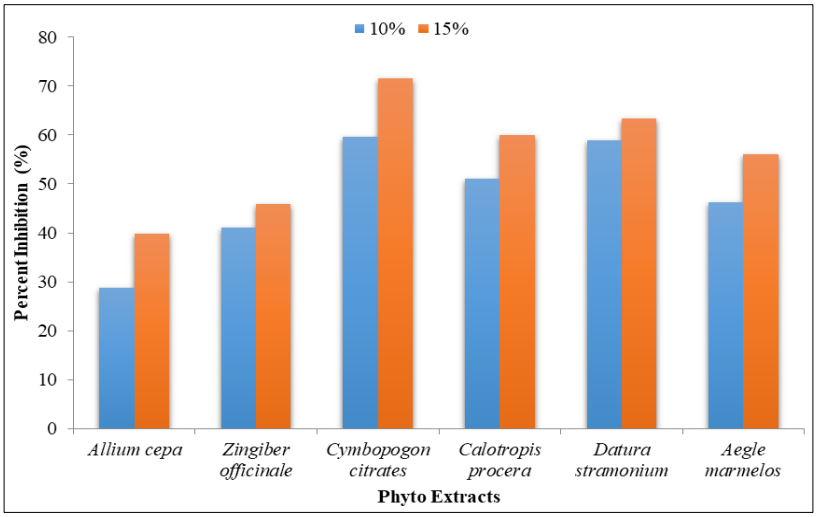
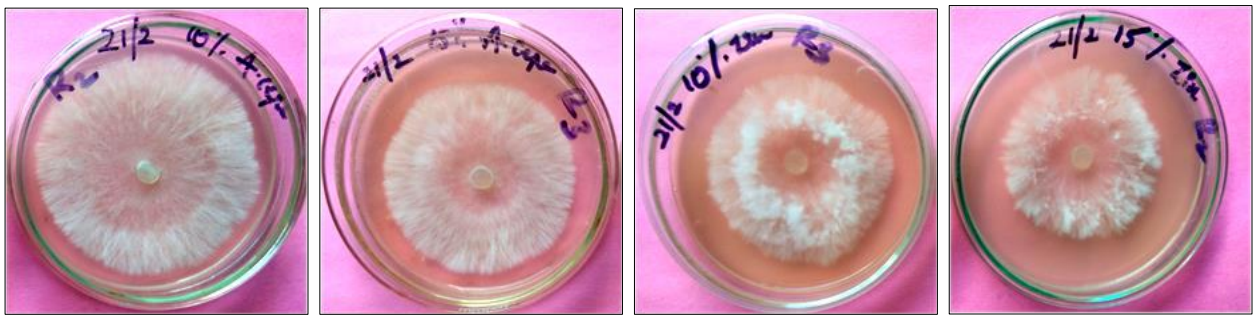
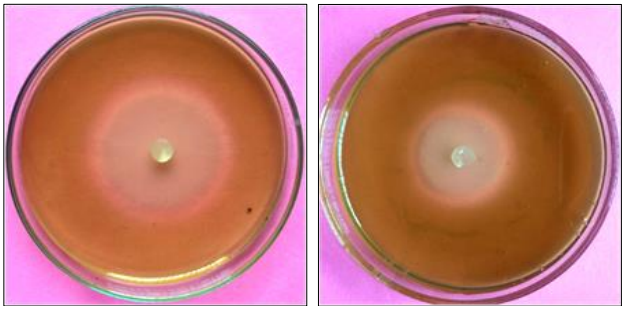


Fig 1: *In vitro* evaluation of different phyto extracts on mycelial growth of *Sclerotium rolfsii*

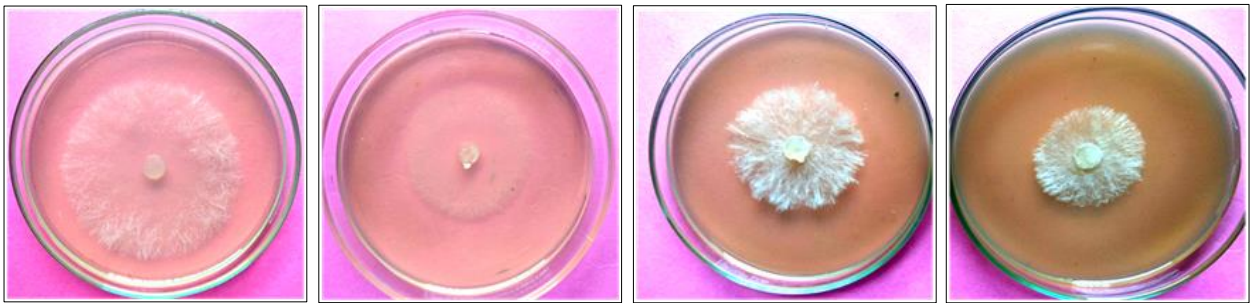


10% *Allium cepa* 15% *Allium cepa* 10% *Zingiber officinale* 15% *Zingiber officinale*



10% *Cymbopogon citrates* 15% *Cymbopogon citrates*

Plate 1: Effect of *Allium cepa*, *Zingiber officinale* and *Cymbopogon citrates* on mycelial growth of *Sclerotium rolfsii*



10% *Calotropis procera* 15% *Calotropis procera* 10% *Datura stramonium* 15% *Datura stramonium*

15% *Aegle marmelos* 15% *Aegle marmelos* Control

Plate 2: Effect of *Calotropis procera*, *Datura stramonium* and *Aegle marmelos* on mycelial growth of *Sclerotium rolfsii*

Summary and Conclusion

Among the six different phyto extracts tested against *Sclerotium rolfsii*, Lemon grass (*Cymbopogon citrates*) was found to be effective against fungus at both concentration showing maximum inhibition rate of 71.66% at 15% followed by Datura (*Datura stramonium*) and Calotrophis (*Calotropis procera*) with 63.33 and 60.00% respectively at higher concentration. It was observed that with increase in botanical concentration, inhibition percent declined significantly. Onion (*Allium cepa*) was ranked last in inhibiting fungus with only 39.77% followed by Zinger (*Zingiber officinale*) with 45.90% inhibition rate.

Utilizing locally available botanical extracts and specific concentration of particular chemical can cut the cost of cultivation. This can also deduct the residual effect on soil and human health. In this way, chickpea growers can obtain higher yields by reducing the infection of *Sclerotium rolfsii* responsible for collar rot disease.

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